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EFFECTS OF GLYPHOSATE ON METABOLISM OF PHENOLIC COMPOUNDS
I. INDUCTION OF PHENYLALANINE AMMONIA-LYASE ACTIVITY IN DARK-GROWN MAIZE ROOTS

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SUMMARY

The herbicide glyphosate, *N*-(phosphonomethyl)glycine, induced phenylalanine ammonia-lyase (PAL) activity in the roots of dark-grown maize seedlings. This enzyme induction was shown to precede glyphosate-reduced fresh weight gain in the roots by 24—48 h. Feeding aromatic amino acids with glyphosate further inhibited growth while slightly lowering glyphosate-enhanced PAL activity. Soluble protein levels in the enzyme preparations were not affected by glyphosate. These results are discussed in terms of possible glyphosate mode of action through stimulating biosynthesis of growth-inhibiting phenolics.

INTRODUCTION

One hypothesized mode of action of the herbicide *N*-(phosphonomethyl)-glycine, commonly known as glyphosate, is inhibition of aromatic amino acid biosynthesis [10,11]. Such a primary effect would slow or halt protein synthesis, resulting in the slowed or halted growth associated with glyphosate treatment. Haderlie et al. [8] have shown that in carrot cell cultures, free pools of aromatic amino acids are not reduced by growth-reducing glyphosate levels. They concluded that synthesis of aromatic amino acids is not specifically inhibited by glyphosate. Thus, reversal of glyphosate effects by feeding aromatic amino acids, as demonstrated in Haderlie's and other systems [7,8,10,11], has not been fully explained. In at least one case [3,9], induction of phenylalanine

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Abbreviations: PAL, phenylalanine ammonia-lyase.

ammonia-lyase (PAL) activity has been shown to reduce aromatic amino acid pool levels sufficiently to reduce growth. These effects could be reversed by feeding phenylalanine. This and the glyphosate results suggested that glyphosate could have an effect on growth through induction of PAL activity. In the present work we show glyphosate to have a stimulatory effect on PAL activity prior to its effect on growth, indicating a possible causal relationship.

MATERIALS AND METHODS

Seeds of *Zea mays* L. cv. Pioneer 3369A were grown in 2 mM $CaSO_4$ at 25°C in complete darkness as previously described [5]. Watering, chemical addition, and harvesting were performed under dim green light. Seedlings were treated with the free acid of glyphosate with or without aromatic amino acids by transferring seedlings to 2 mM $CaSO_4$ solutions with the indicated levels of treatment compound(s). Seedlings remained in the test solutions until harvest. Harvesting was as previously described [6]. PAL from roots and mesocotyls was extracted, purified and assayed as previously described (4—6). Glyphosate up to 10^{-3} M had no in vitro effect on PAL activity. Total hydroxyphenolics were assayed by the improved Folin method of Singleton and Rossi [12]. Error bars are standard errors of means of 3—4 experiments.

RESULTS AND DISCUSSION

When 3-day-old seedlings were transferred to 10^{-3} M glyphosate solutions, significant differences between control and glyphosate root fresh weights were not apparent until 72 h (Fig. 1). However, large significant differences in PAL activity, per gram of fresh weight and per plant root system, occurred after only 24 h of glyphosate treatment (Figs. 2A and B). Significant differences in specific activity appeared after 48 h of treatment (Fig. 2C). Soluble protein precipitated with the enzyme was not affected by glyphosate on either a fresh weight basis (Fig. 3A) or a per root basis (Fig. 3B).

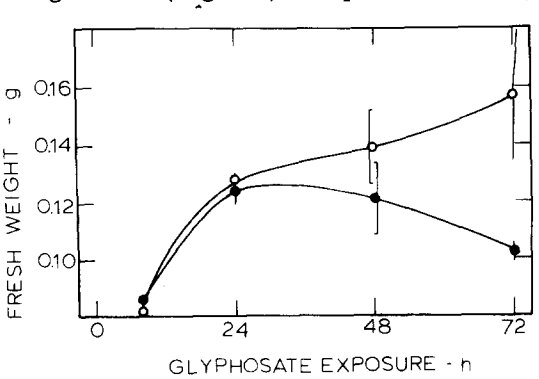

Fig. 1. Changes in fresh weight of roots of intact, dark-grown, 3-day-old, maize seedlings after transfer to 10^{-3} M glyphosate (•) or water (○).

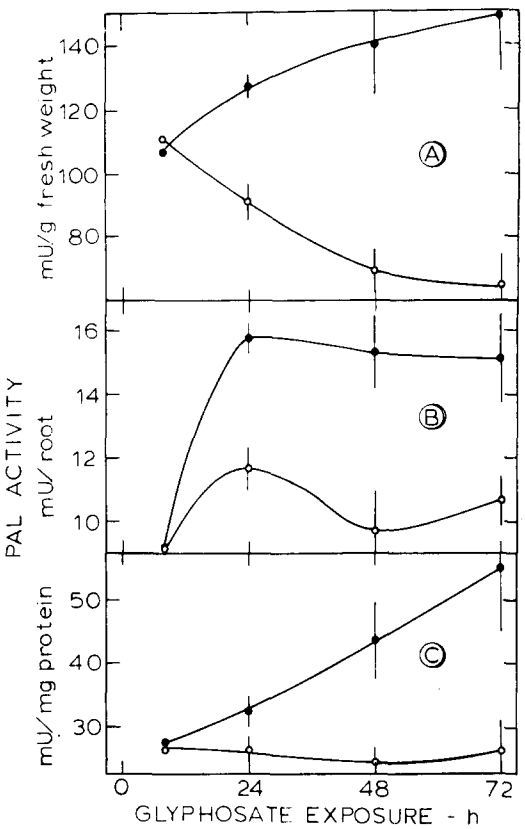

Fig. 2. Changes in PAL activity in roots of intact, dark-grown, 3-day-old, maize seedlings after transfer to 10^{-3} M glyphosate (•) or water (○): (A) PAL activity/g fresh weight; (B) PAL activity /plant root system; (C) PAL activity/mg protein in enzyme extract.

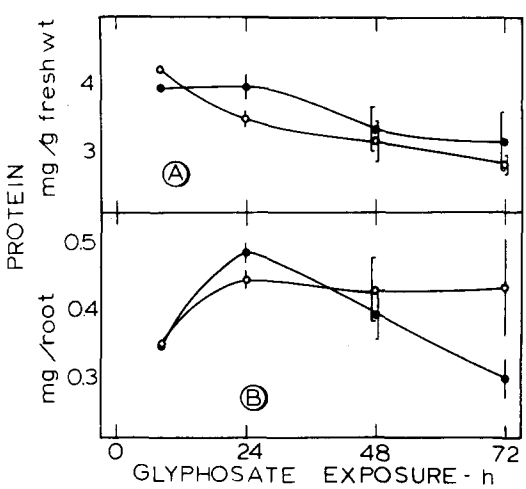

Fig. 3. Changes in protein in PAL extracts from roots of intact, dark-grown, 3-day-old, maize seedlings after transfer to 10^{-3} M glyphosate (•) or water (○): (A) mg protein/g fresh weight; (B) mg protein/plant root system.

TABLE I

EFFECTS OF GLYPHOSATE WITH AND WITHOUT PHENOLIC AMINO ACIDS ON PAL ACTIVITY, SOLUBLE PROTEIN AND FRESH WEIGHT OR DARK-GROWN MAIZE SEEDLING ROOTS

Treatment	Time after transfer[a]	PAL activity			Soluble protein		Fresh wt. g/root
		mU/mg protein	mU/g fresh wt.	mU/root	mg/g fresh wt.	mg/root	
Control	5	8.48	16.6	3.56	2.08	0.449	0.215
	8	4.43	3.9	1.82	0.88	0.411	0.467
Glyphosate (5×10^{-4} M)	5	17.87	25.0	6.62	1.42	0.361	0.206
	8	19.37	24.9	5.69	1.29	0.294	0.229
Glyphosate, phenylalanine, tyrosine, and tryptophan (5×10^{-4} M each)	5	11.16	19.65	3.63	1.73	0.323	0.191
	8	11.63	14.43	2.07	1.24	0.178	0.144

[a] Transfer was at 2 days.

These data suggest a causal relationship between glyphosate induction of PAL activity and glyphosate-inhibited fresh weight gain. Our results do not indicate that the mechanism of this postulated mode of action is through reduction of protein synthesis by depletion of the phenolic amino acid pool. An alternative hypothesis is that glyphosate-induced PAL activity results in enhanced levels of growth-inhibiting phenolic compounds.

The data of Table I support the view that growth is inhibited by glyphosate-induced phenolics in our system. Feeding aromatic amino acids along with 5×10^{-4} M glyphosate resulted in intermediate PAL induction when compared to glyphosate alone and the water control. Nevertheless, growth was inhibited more than with glyphosate alone. Also, preliminary results show that levels of total hydroxyphenolics in glyphosate-treated roots relative to controls begin to increase after 1 day, reaching about 25% enhancement after 3 days of treatment. Increases in PAL activity have been correlated with anthocyanin [4] and total phenolic [13] synthesis in maize seedlings. Generally, increased PAL activity is thought to result in enhanced phenolic production [2]. Our data, in the light of accepted PAL theory, do not support the theory that glyphosate inhibits aromatic amino acid biosynthesis [10,11].

However, duckweed [10] and *E. coli* [11] may respond differently to glyphosate than maize does. All glyphosate mode of action data from non-aquatic higher plants [1,7,8] of which we are aware lends no support to the inhibition of aromatic amino acid hypothesis. However, none of these studies has dealt with the enzymology or turnover of phenolic amino acids. In previous work with roots of maize seedlings, light induced a two-fold increase in PAL activity which resulted in approx. 50% reduction in the free pool of phenylalanine, while the turnover rate of phenylalanine increased three- to six-fold [6].

No significant effects of glyphosate were found on growth or on PAL activity of maize seedling mesocotyls up to 72 h after treatment. This may be due to the fact that glyphosate is poorly transported from roots to mesocotyls of maize seedlings as reported by Haderlie [7]. He could not detect transport of label until 4 days of root exposure.

In conclusion, we believe that our data, in the context of that of others, indicate that glyphosate could inhibit root growth during maize seedling development by PAL induction accompanied by an increase of growth-inhibiting phenolics. However, the relationship between PAL activity and growth that we have shown may only be a correlation, since many stress conditions have been shown to induce PAL [14]. Also, the effect that we have demonstrated could be one of several growth-limiting secondary effects of glyphosate. Brecke [1] has shown that glyphosate alters membrane properties within 1 h after treatment, suggesting a primary effect. In systems in which glyphosate action is reversed by feeding aromatic amino acids [10,11] glyphosate may act through induction of PAL with resulting aromatic amino acid pool depletion and decreased protein synthesis. We are currently investigating this possibility.

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